Paper No. 25

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte IAN HOLMGREN and ANN-MARI SVENNERHOLM

Appeal No. 1999-2634
Application No. 08/108,606

ON BRIEF

Before WINTERS, SCHEINER, and MILLS, Administrative Patent Judges.

MILLS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 7-11 and 13-19, which are all of the claims pending in this application.

Claims 7, 9, 11, 13 and 15 are illustrative of the claims on appeal and read as follows:

7. A method for producing a formalin-killed E. coli bacterial strain for use in a vaccine against enteric infection caused by E. coli bacteria in humans comprising the steps of:

providing at least one E. coli bacterial strain expressing colonization factor antigens on the surface of said bacteria,

growing said E. coli bacterial strain in a liquid culture medium with vigorous agitation to a predetermined density,

harvesting said E. coli bacterial strain,

resuspending said harvested E. coli bacterial strain in saline,

adding formalin to said harvested, resuspended bacterial strain to a final concentration of 0.2M formaldehyde,

incubating said formalin-treated E. coli bacterial strain at 37°C under conditions of continuous agitation for about 2 hours,

further incubating said formalin-treated bacterial strain at 4°C for between about 24 hours and about 48 hours, thereby obtaining a formalin-killed E. coli bacterial strain and,

collecting said formalin-killed E. coli bacterial strain.

9. The method according to claim 7, wherein said liquid culture medium comprises 1% (w/v) casamino acids, 0.15% (w/v) yeast extract, 0.4 mM $MgSo_4$,0.04mM $MgCl_2$, and deinozed water at pH 7.4, and said growing step is conducted with vigorous agitation at about 37°C for at least 4-6 hours before said harvesting step.

11. The method according to claim 7 further comprising adding an acid-neutralizing buffer.

13. A method of prevention [sic of] an enteric infection caused by enterotoxigenic E. coli bacteria in humans comprising administrating an appropriate amount for preventing said infection of a vaccine comprising at least one formalin-inactivated E. coli strain expressing colonization factor antigens and further having substantially preserved antigenic and hemagglutinating properties of said colonization factor antigens.

15. The method of claim 13, wherein said vaccine further comprises cholera toxin b-subunit.

The prior art references relied upon by the examiner are:

Myers 4,338,298 July 6, 1982

Evans, D.J., et al (Evans 1), "Immunoprotective oral whole cell vaccine for enterotoxigenic *Escherichia coli* diarrhea prepared by in situ destruction of chromosomal and plasmid DNA with colicin E2," FEMS Microbiology and Immunology, Vol. 47, pp. 9-18 (1988)

Svennerholm, A.M., et al. (Svennerholm), "Development of oral vaccines against enterotoxinogenic *Escherichia coli* diarrhoea," Vaccine, Vol. 7, No. 3, pp. 196-198 (1989)

Solderlind, et al. (Soderlind), "Effect of Parenteral Vaccination of Dams on Intestinal *Escherichia coli* in Piglets with Diarrhea," Infection and Immunity, Vol. 36, pp. 900- 906 (1982)

Gregory, et al (Gregory), "Lamb model in the study of immunity to enteropathogenic *Escherichia coli* infections," American Journal of Veterinary Research, Vo. 44, p. 2073 (1983)

Evans, et al. (Evans 2), "Hemmagglutination of Human Group A Erythrocytes by Enterotoxigenic *Escherichia coli* Isolated from Adults with Diarrhea: Correlation with Colonization Factor," Infection and Immunity, Vol. 18, p. 330 (1977)

Evans, et al. (Evans 3), "Administration of Purified Colonization Factor Antigens (CFA/I, CFA/II) of Enterotoxigenic *Escherichia coli* to Volunteers," Gastroenterology, Vol. 87, pp. 934 (1984)

Claim Grouping

According to appellants, the claims stand or fall together in the following groups: Group 1, claims 7, 8, 10, 11 and 16; Group 2, claim 9; Group 3, claim 11; Group 4, Claims 13 and 14; Group 5, claim 17 and Group 6, claims 15, 18 and 19. (Brief, page 5). We decide this appeal on the basis of claim 7 as representative of claims 7-11, 16 and 17; and Claim 13 as representative of the claims 13-15, 18 and 19. In re Young, 927 F.2d 588, 590, 18 USPQ2d 1089, 1091 (Fed. Cir. 1991).

Grounds of Rejection

Claims 7, 8, 10 and 13-19 stand rejected under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers.

Claim 9 stands rejected under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers in further view of Evans 2.

Claim 11 stands rejected under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers in further view of Evans 3.

DISCUSSION

In reaching our decision in this appeal, we have given consideration to the appellants' specification and claims, to the applied prior art references, and to the respective positions articulated by the appellants and the examiner.

Rather than reiterate the conflicting viewpoints advanced by the examiner and the appellants regarding the noted rejection, we make reference to the examiner's Answer for the examiner's reasoning in support of the rejection, and to the appellants' Brief for the appellants' arguments thereagainst. As a consequence of our review, we make the determinations which follow.

35 U.S.C. § 103

Claims 7, 8, 10, 13-16 and 17-19 stand rejected under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers. Claim 9 stands rejected under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and

Soderlind or Gregory and Myers in further view of Evans 2. Claim 11 stands rejected under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers in further view of Evans 3.

In rejecting claims under 35 U.S.C. § 103, the examiner bears the initial burden of presenting a prima facie case of obviousness. See In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). A prima facie case of obviousness is established when the teachings from the prior art itself would appear to have suggested the claimed subject matter to a person of ordinary skill in the art. In re Bell, 991 F.2d 781, 783, 26 USPQ2d 1529, 1531 (Fed. Cir. 1993). An obviousness analysis requires that the prior art both suggest the claimed subject matter and reveal a reasonable expectation of success to one reasonably skilled in the art. In re Vaeck, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). With this as background, we analyze the prior art applied by the examiner in the rejection of the claims on appeal.

Claim 7

According to the examiner, (Paper No. 16, page 4)

> Evans et al teach a method for producing an *E. coli* bacterial strain expressing colonization factor antigens (CFA/1) for use as an oral vaccine against enteric infection which has the following steps: (1) growing the *E.*

coli bacterial strain expressing the colonization factor antigens in a liquid culture medium to a predetermined density of about A_{640} of about 0.9: (2) harvesting the *E. coli* bacterial strain; (3) resuspending the bacterial strain in water; (4) treating the cells with colicin E2 at 37°C while agitating; (5) harvesting and resuspending the bacterial strain in saline (p 10, column 2, paragraph 2). Evans et al disclose adding a pharmaceutically acceptable diluent to the bacterial cells. ... Evans et al teach the oral administration of the inactivated vaccine. ...Evans et al does not teach treating the bacterial strain with formalin. Evans et al does not teach the administration of the vaccine with Cholera toxin-b subunit (CTB).[1] Evans also does not specifically teach vigorous agitation during the entire growing step nor

[1] Although the examiner suggests that Evans does not does not teach the administration of the vaccine with Cholera toxin-b subunit (CTB) we note Evans 1 does teach in the Summary on page 9, that the oral vaccine testing in Evans was (ST+LT+; 078:H11:CFA/1). We further note, Svennerholm indicates that heat labile enterotoxin (LT) cross reacts with the beta subunit of cholera toxin. Svennerholm, page 196.

does Evans et al teach growing the E. coli to a predetermined density of about 10^{10} bacteria/ml.

The examiner relies on Svennerholm for the teaching that candidate vaccines for enterotoxin-producing bacteria include bacteria which are inactivated with formalin treatment or colicin E2 treatment. Svennerholm teaches that the inactivation of bacteria with mild formalin treatment causes complete killing of the bacteria without significant loss of the antigenicity of the different CFAs and O antigens. In addition, Svennerholm teach that both anti-enterotoxin and anti-colonization factor antibodies can, independently of each other, protect against experimental enterotoxin-producing E. coli infection and when present together, these antibody specificities cooperate synergistically in protecting against infection, and that for maximal efficacy an ETEC (enterotoxin producing Escherichia coli) vaccine should ideally invoke an immune response that would effectively interfere with both colonization and toxin action. Paper No. 16, page 5.

Soderlind is relied on for its disclosure of commercially available formalin-killed E. coli vaccine containing 12 strains of E. coli. Gregory discloses bacterins prepared from formalin-killed E. coli for the vaccination of sheep. Gregory also teaches that the K99 antigen is a fimbrial adhesive antigen which facilitates colonization of mucosal surfaces. Myers is relied on for its disclosure of the growth of E. coli strains which contain K99 antigens in a suitable growth medium with vigorous shaking for twenty to

twenty four hours and treating the E. coli with 0.2 to 0.4% formalin to prepare a killed E. coli vaccine.

The examiner summarizes (Paper No. 16, pages 5-7)

> It would have been obvious to one of ordinary skill in the art to substitute formalin treatment for the colicin E2 taught by Evans et al because a formalin-killed vaccine causes complete killing of bacteria without significant loss of the antigenicity of the different CFAs and O antigens and would be an alternative vaccine to the colicin E2 treated cells as taught by Svennerholm et al. In addition the use of formalin to produce killed *E. coli* vaccines is a common technique in the art as exemplified by the preparations taught by Soderlind et al and Gregory et al. It would have been obvious to use 0.2 M formaldehyde because this concentration is equivalent to 0.2% formalin which is used to kill *E. coli* for use as a vaccine as taught by Myers. ... It would have been obvious to resuspend the harvested strain in saline instead of water prior to formalin treatment because saline is a more physiologically compatible solution. It would have been obvious to optimize the timing of the treatment of the cells with formalin because such optimization would constitute routine experimentation and be within the skill of the ordinary artisan. **It would also have been obvious to further incubate the formalin-treated bacterial strains at 4°C in order to prevent contamination of the culture or denaturation of the bacterial antigens prior to collecting them for use in the vaccine.** It would have been obvious to grow *E. coli* with vigorous shaking because this is a routine condition for growing *E. coli* and may be successfully used for preparing a killed vaccine in which the E. coli contain colonization fimbrial antigens as taught by Myers et al. Optimization of the density of the *E. coli* in the culture medium prior to harvesting would be considered routine in the art and would be withing the skill of the ordinary artisan. It would have been obvious to orally administered an appropriate among of the formalin-inactivated vaccine having CFA's with antigenic and hemagglutinating properties and CTB to prevent an enteric infection caused by enterotoxigenic *E. coli* bacteria in humans because an orally administered inactivated vaccine expressing CFA/I provided protection in humans as taught by Evans et al, and Svennerholm et al specifically teach the advantages of adding CTB to an orally administered ETEC vaccine. Therefore, one of skill in the art would expect that a formalin-inactivated *E. coli* strain expressing CFA's

> administered with CTB would provide protection when administered as a vaccine as taught by Svennerholm et al. [Emphasis added.]

"[P]atentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of the argument." In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); In re Piasecki, 745 F.2d 1468, 1471-72, 223 USPQ 785, 787 (Fed. Cir. 1984). Findings of fact and conclusions of law must be made in accordance with the Administrative Procedure Act, 5 U.S.C. 706 (A), (E) (1994). See, Zurko v. Dickinson, 527 U.S. 150, 158, 119 S.Ct. 1816, 1821, 50 USPQ2d 1930, 1934(1999). In addition upon judicial review, findings of fact relied upon in making the enablement rejection must be supported by substantial evidence within the record. See In re Gartside, 203 F.3d 1305,1315, 53 USPQ2d 1769, 1775 (Fed. Cir. 2000).

In the present case, with respect to claims 7-11,16 and 17, we find the examiner has failed to establish a prima facie case of obviousness. Although the examiner suggests it would have been obvious to further incubate the formalin-treated bacterial strains at 4°C in order to prevent contamination of the culture or denaturation of the bacterial antigens prior to collecting them for use in the vaccine, the examiner has failed to provide evidence, such as in the form of a patent, reference material or publication to support this position. Patent examiners, in relying on what they assert to be general knowledge to negate patentability on the ground of obviousness, must articulate that knowledge and place it of record, since examiners are presumed to act from the

viewpoint of a person of ordinary skill in the art in finding relevant facts, assessing the significance of prior art, and making the ultimate determination of the obviousness issue. Failure to do so is not consistent with either effective administrative procedure or effective judicial review, examiners cannot rely on conclusory statements when dealing with particular combinations of prior art and specific claims, but must set forth the rationale on which they rely. See In re Lee, 277 F.3d 1338, 1343-1344, 61 USPQ2d 1430, 1433-1434 (Fed. Cir. 2002). Thus, it is improper to rely on the "common knowledge and common sense" of a person of ordinary skill in art to find an invention obvious over a combination of prior art references, since the factual question of motivation to select and combine references is material to patentability, and cannot be resolved on subjective belief and unknown authority. In re Lee, 277 F.3d 1338, 1343-1344, 61 USPQ2d 1430, 1433-1434 (Fed. Cir. 2002). Therefore, while not commenting on whether one of ordinary skill in the art would have found it obvious to further incubate the "formalin-treated bacterial strain at 4°C for between about 24 hours and about 48 hours, thereby obtaining a formalin-killed E. coli bacterial strain," we are constrained to find the examiner has failed to establish a prima facie case of obviousness for failing to provide evidence of this fact.

The rejection of claim 7, its dependent claims 8-11,16 and independent claim 17, including the same process step, is reversed.

Claims 13 and 15

Claim13 is directed to a method of prevention of an enteric infection caused by enterotoxigenic E. coli bacteria in humans comprising administrating an appropriate amount for preventing said infection of a vaccine comprising at least one formalin-inactivated E. coli strain expressing colonization factor antigens and further having substantially preserved antigenic and hemagglutinating properties of said colonization factor antigens. Claim 15 further requires that the vaccine of claim 13 further comprises cholera toxin b-subunit.

The examiner relies on Svennerholm for the teaching that candidate vaccines for enterotoxin-producing bacteria include bacteria which are inactivated with formalin treatment or colicin E2. Svennerholm teaches that the inactivation of bacteria with mild formalin treatment causes complete killing of the bacteria without significant loss of the antigenicity of the different CFAs (colonization factor antigens) and O antigens. In addition, Svennerholm teach that both anti-enterotoxin and anti-colonization factor antibodies can, independently of each other, protect against experimental enterotoxin-producing E. coli infection and when present together, these antibody specificities cooperate synergistically in protecting against infection, and that for maximal efficacy an ETEC vaccine should ideally invoke an immune response that would effectively interfere with both colonization and toxin action. Paper No. 16, page 5.

Svennerholm, page197, column 3 to page 198 column 4, indicates that immunization of both animals and human volunteers with a colicin treated E. coli strain

which was CFA/I positive evoked partial protection against challenge with CFA homologous as well as CFA-heterologous ETEC strains. Because Svennerholm suggests that colicin treatment is an alternative method to mild formalin treatment to inactivate ETEC bacteria, it would have been obvious to one of ordinary skill in the art at the time of the present invention to substitute mild formalin treatment for the colicin treatment to inactivate CFA positive ETEC cells with an expectation of success that it would provide for a protective oral vaccine. We also find Evans 1, 2, Soderlind and Gregory to be cumulative and supportive of the efficacy of formalin treated ETEC vaccines.

Where the prior art, as here, gives reason or motivation to make the claimed invention, the burden then falls on an appellants to rebut that prima facie case. Such rebuttal or argument can consist of any other argument or presentation of evidence that is pertinent. In re Dillon, 919 F.2d 688, 692-93, 16 USPQ2d 1897, 1901 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991).

In response to the examiner's arguments, appellants argue (Brief, pages 7-8) that "Svennerholm clearly indicate that efficacious anti-ETEC vaccines were still theoretical. ... [A]ccordingly, one of ordinary skill in the art would have recognized that success could only be measured by clinical data which had not yet been acquired at the date of publication. Thus, appellants argue that Svennerholm provides no reasonable expectation of success of obtaining an efficacious ETEC vaccine. Brief, page 8.

In response, it is the position of the examiner that (Answer, pages 5-6)

> Svennerholm does more than invite experimentation but teaches the administration of oral vaccines for protection against enterotoxin-producing *E. coli*. Evans et al and Svennerholm et al teach the administration of an oral ETEC vaccine, it is maintained that the combination of this art is appropriate despite the fact that the vaccines of Meyers, Soderlind and Gregory are administered parenterally because the ordinary artisan would have had a reasonable expectation of success using the teachings of Myers, Soderlind and Gregory in the method of Evans et al in light of Svennerholm et al because Myers, Soderlind and Gregory are analogous art as they focus on vaccines against enterotoxin-producing *E. coli* and each reference defines conditions which provide insight to the nature of the antigens of enterotoxin-producing *E. coli* which would result in the ordinary artisan having a reasonable expectation of success of attaining a vaccine which is optimized against *E. coli* infection.

We agree with the examiner that Soderlind, alone or in combination with Evans 1 and 2, Soderlind, Gregory and Myers provide the requisite reason, suggestion or motivation to substitute mild-formalin treated bacteria for colicin treated bacteria for preparation of an ETEC vaccine, and the reasonable expectation of success. The reasonable expectation of success is particularly supported by Evans 1 disclosure of a successful whole cell ETEC vaccine and evidence of record that mild formalin treatment also provides for a whole cell vaccine.

With respect to separately argued claims 15 and 18, we note particularly the evidence provided in the disclosure of Svennerholm, that the anti-LT immune response is mainly against the B subunit portion of the molecule which cross-reacts immunologically with the B subunits of cholera toxin, and the recognition that candidate vaccines should thus, contain a combination of bacterial cell-derived and toxin-derived antigens. Svennerholm, page 197.

In view of the discussion above, we find that the examiner has presented a prima facie case of obviousness of the invention of claims 13-15, 18 and 19 which has not been overcome with appropriate evidence by appellants.

CONCLUSION

The examiner's rejection of claims 7, 8, 10 and 16-17 under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers; the rejection of claim 9 under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers in further view of Evans 2; and the rejection of claim 11 under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers in further view of Evans 3 are reversed. The rejection of claims 13-15, 18 and 19 under 35 U.S.C. § 103 over Evans 1 in view of Svennerholm, and Soderlind or Gregory and Myers is affirmed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED-IN-PART

SHERMAN D. WINTERS Administrative Patent Judge	) BOARD OF PATENT
TONI R. SCHEINER Administrative Patent Judge	) APPEALS AND
DEMETRA J. MILLS Administrative Patent Judge	) INTERFERENCES

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